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Conformationally biased P3 amide replacements of β-secretase inhibitors

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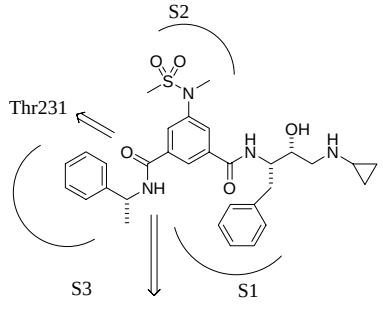
Abstract—We have synthesized and evaluated a series of conformationally biased P3 amide replacements based on an isophthalamide lead structure. The studies resulted in the identification of the β-secretase inhibitor **7m** which has an in vitro IC_{50} = 35 nM. The synthesis and biological activities of these compounds are described.

Alzheimer's disease (AD) is a neurodegenerative disease of the brain that is characterized by the progressive formation of insoluble amyloid plaques and fibrillary tangles.[1] These plaques and tangles consist primarily of aggregated Aβ, a 40–42 amino acid peptide that is formed by the proteolytic processing of β-amyloid precursor protein (βAPP) by two enzymes, β- and γ-secretase.[2] The rate-limiting enzyme in this catabolic process is β-secretase (β-site APP cleaving enzyme or BACE-1), a novel aspartyl protease whose identity was uncovered only a few years ago.[3] As such, β-secretase inhibition is considered an attractive therapeutic target for the treatment and prevention of AD.



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5-Substituted isophthalamides, as represented by structure **1**, emerged as an early lead class that was investigated for inhibition of β-secretase.[4] Inhibitor **1** possessed the desirable properties of both good in vitro (IC_{50} = 15 nM) and cell-based potencies (IC_{50} = 29 nM) against BACE-1 and was found to be 10-fold selective versus the homologous enzyme BACE-2. However, problems associated with its pharmacodynamic properties precluded it as a candidate for advancement. A main concern was that **1** was found to be a substrate of P-glycoprotein (P-gp) transport. Since a β-secretase inhibitor must inevitably exhibit activity intracranially, it is imperative that it be able to cross the blood–brain barrier and achieve sustainable drug levels. It is generally accepted that a greater number of functional groups with a close proximity of hydrogen bond donors/acceptors to each other, i.e., amide bonds, increase the likelihood that a compound will act as a P-gp substrate.[5] This said, we became interested in the replacement of the bulky α-methylbenzamide P3 appendage present in **1** with a smaller, non-amide pharmacophore.

The co-crystal structure of β-secretase with a statin derivative, based on a peptidic fragment of APP, was

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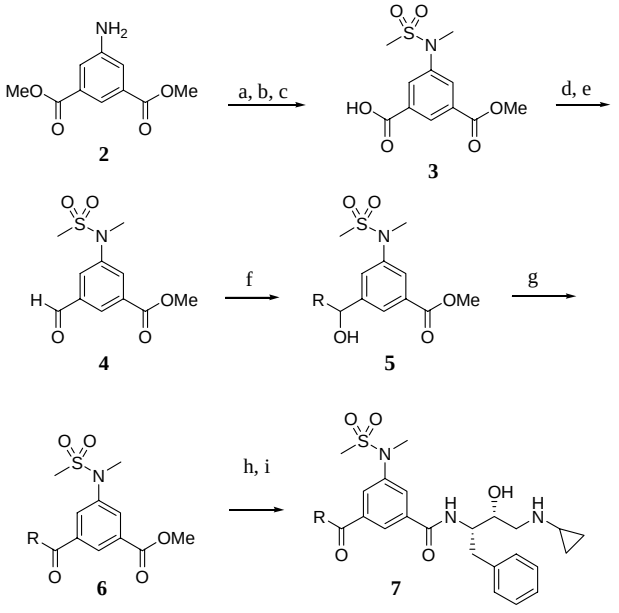
first published in 2000.[6] In general, APP, or peptide fragments thereof, are arranged in the standard zig-zag conformation in the BACE-1 active site. However, the catalytic domain of BACE-1 in comparison with those of other aspartyl proteases (e.g., pepsin, HIV protease) is more open and less hydrophobic. The S3 site is one of the more hydrophobic domains in the active site and its preferred amino acid subunit is valine.[7] Within the S3 domain of inhibitor **1** the corresponding amide linker is involved in two distinct hydrogen bonding interactions, namely NH to GLY230 and C=O to THR231. We hypothesized that this P2/P3 amide group serves mainly to orient the peptide backbone for presentation of the amino acid side chains and that amide removal could be achieved without abrogating activity, as long as the substrate is conformationally biased to orient the side chain toward the S3 pocket.

The truncated methyl amide **7a** was chosen as a starting point to investigate the feasibility of removing the amide functionality. Direct replacement of the amide nitrogen with a methylene group (**7b**), thereby removing the NH hydrogen bonding contact, resulted in only a 2-fold loss in potency. This result was encouraging since it demonstrated that amide removal was tolerated without significant loss of activity. Next we focused on regaining potency toward the enzyme by accessing the S3 binding domain with a lipophilic appendage.

The general synthetic scheme for the synthesis of compounds **7b**–**f** is shown in Scheme 1. Mesylation of commercially available dimethyl 5-amino isophthalate using methanesulfonyl chloride in pyridine gave the desired sulfonanilide in high yield. N-methylation was accomplished with sodium hydride and methyl iodide which was followed by saponification to give benzoic acid **3**.

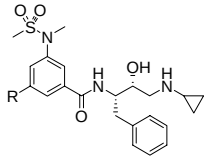


Scheme 1. Reagents: (a) MsCl, pyridine, CH_2Cl_2, 75%; (b) NaH, MeI, DMF, 98%; (c) 0.1 N LiOH, THF/H_2O, 67%; (d) EDC, HOBT, *N*,*O*-dimethylhydroxylamine HCl, $NaHCO_3$, 81%; (e) DIBAL-H, 94%; (f) RMgX; (g) MnO_2, CH_2Cl_2; (h) 1 N NaOH, THF/MeOH/H_2O (i) BOP, hydroxyethyldiamine, diisopropylethylamine.

Aldehyde formation was achieved by the two-step process of Weinreb amide formation and subsequent Dibal reduction. Addition of the appropriate Grignard reagent to aldehyde **4** produced benzylic alcohols **5** that could be carried through the scheme or oxidized to the corresponding ketones **6** using manganese oxide. Hydrolysis and BOP mediated amide coupling to the dihydrochloride salt of (2*R*,3*S*)-*N*-1-cyclopropyl-2-hydroxy-4-phenylbutane-1,3-diamine[4] afforded the target inhibitors **7**(**a**–**n**). It should be noted that secondary Grignard reagents that contained a β-hydrogen (i.e., cyclopentyl, isopropyl) displayed a competitive, and often chemoselective, 1,2-reduction of aryl aldehyde **4** resulting in depressed yields for compounds **7i**–**k**.

The general schemes outlined above allowed access to P3 substituents and the IC_{50} values for these inhibitors versus BACE-1 are shown in Table 1.[8] The results ob-

Table 1. SAR of P3 amide replacements



Compound	R	IC_{50} (nM) *
7a		980
7b		1500
7c		1400
7d		5500
7e		98
7f		5900
7g		1300
7h		4630
7i		450
7j		240
7k		180
7l		730
7m		35
7n		420

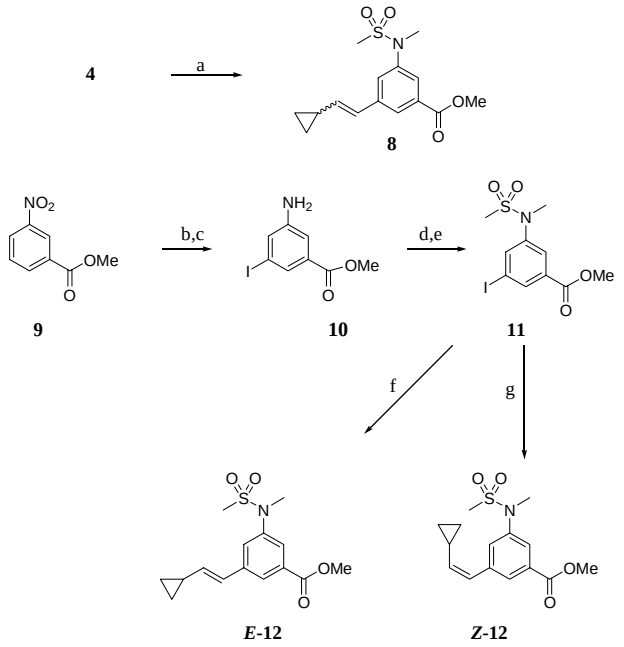
*IC_{50}'s reported as $n = 1$.

tained from deoxybenzoin **7c**, which was predicted to access the S3 domain, were less than anticipated and only a nominal change in potency was achieved. It was reasoned that significant amounts of enol tautomers in solution led to either a non-optimal binding conformation or unfavorable interactions with the enol hydroxyl group. Reduction of the ketone **7c** to alcohol **7d** effectively prevented enolization and resulted in further reduction in potency. We assumed the hydroxyl group presented an unfavorable interaction with the enzyme, so we focused on reducing the flexibility of the benzyl group by synthesizing benzophenone **7e** and a 15-fold increase in potency was obtained. Intriguingly, the reduced benzophenone **7f** resulted in a loss of potency. This result coupled with that of **7d** implies that loss of activity is most likely due to an unfavorable interaction of the benzyl hydroxyl group with the enzyme. Analysis of the co-crystal structure of **7e** in the BACE-1 active site clearly showed the phenyl ring oriented directly into the S3 pocket and the benzophenone carbonyl oxygen pointing toward the solvent front.

In order to validate the 'orienting' effects of the benzophenone carbonyl group, which seemed to lack a hydrogen bonding contact with the enzyme, the isosteric olefin **7g** was synthesized. While we anticipated this compound to display a comparable binding conformation to **7e**, a 13-fold loss in potency resulted. Nevertheless, this compound exhibited a 4-fold enhancement over the sp^3 analog **7h**. It can be reasoned that selective orientation is a factor that contributes to potency but the ketone oxygen gained additional stabilization through electrostatic contacts, most likely with the solvent boundary.

To further improve the potency of benzophenone **7e**, various aryl replacements were investigated with a goal toward filling the S3 binding domain with the optimal ligand (**7i**–**k**). While molecular modeling predicted cyclopentyl derivative **7k** to have the optimal fit, it was proven experimentally to be slightly less potent than the phenyl analog **7e**. One possible explanation is that π-stacking within the S3 binding pocket could provide the additional increase in binding energy.

The above results established that omission of the P2/P3 amide can be achieved while retaining potency only if the P3 appendage is properly oriented. As such, we turned our attention to an alternative means of orienting the P3 group, that of a geometrically defined internal olefin. We had predicted through modeling a priori that the *cis*-alkenyl isomer (**7m**) would be most conformationally biased to access S3 thereby mimicking the *s-trans* amide bond geometry. Initial attempts to synthesize the desired *E*/*Z* olefins via a Wittig reaction resulted in a 2:1 mixture of *cis*/*trans* isomers that proved to be chromatographically inseparable (Scheme 2, cf. **4** → **8**). In order to access a geometrically pure material, a modified synthetic route that employed iodide **11** was devised (Scheme 2). Methyl 3-nitrobenzoate was iodinated with *N*-iodosuccinimide in triflic acid to produce the iodoarene.[9] The strongly acidic iodination procedure was necessary due to the electron-deficient nature of the aryl ring. While the yield for the reaction was



Scheme 2. Reagents: (a) Cyclopropylmethyl triphenylphosphonium bromide, *n*-BuLi, 46%; (b) TfOH, NIS, 37%; (c) $SnCl_2 \cdot H_2O$, THF/EtOH, 96%; (d) MsCl, pyridine, CH_2Cl_2, 85%; (e) NaH, MeI, DMF, 98%; (f) cyclopropylacetylene, 9-BBN, $PdCl_2 \cdot (dppf)$ CH_2Cl_2, $AsPh_3$, Cs_2CO_3, 73%; (g) cyclopropylacetylene, $InCl_3$, DIBAL-H, Et_3B, $Pd(dba)_2 \cdot CHCl_3$, $P(furyl)_3$, 93%.

modest, 37%, the remaining material was unreacted benzoate. Subsequent reduction of the iodoarene with stannous chloride afforded aniline **10**. The aniline was then mesylated and methylated using the previously described conditions. Access to *trans* isomer **7l** was realized via a Suzuki reaction between **11** and the organoborane derived from the hydroboration of cyclopropyl acetylene resulting in ***E*-12**. Synthesis of the *cis* isomer (**7m**) employed an indium(II) catalyzed cross coupling reaction recently reported by Oshima.[10] In that regard, triethylborane induced hydroindination of cyclopropylacetylene produced the (*Z*)-alkenylindium species which smoothly underwent palladium mediated cross coupling to yield the (*Z*)-alkene ***Z*-12** exclusively in 93% yield. We were pleased to find that the *cis*-olefin **7m** did in fact orient itself in the S3 pocket with excellent binding activity. Figure 1 shows an overlay of the X-ray crystal structure of **1**[11] with a model of **7m**. The figure depicts how the *cis*-alkene effectively mimics the *s-trans* amide binding orientation present in **1**. In contrast, the *trans*-olefin (**7l**) displayed a 20-fold reduction in activity versus the *cis*-olefin. In fact, the *trans*-olefin (**7l**) was even less favorable than the fully saturated analog **7n** reinforcing the effect that conformational bias has on potency.

As noted previously, compound **1** had significant pharmacokinetic liabilities associated with it. While **1** was considered to be a severe P-gp substrate, the apparent permeability (Papp) was so low (0.6×10^{-6} cm/s) that accurate P-gp efflux values were indeterminable. In contrast, while compounds such as **7m**, containing an olefinic linkage, were still found to be moderate substrates for P-gp efflux (B/A-A/B mdr1a = 8) the Papp values (14×10^{-6} cm/s) were significantly increased as to permit

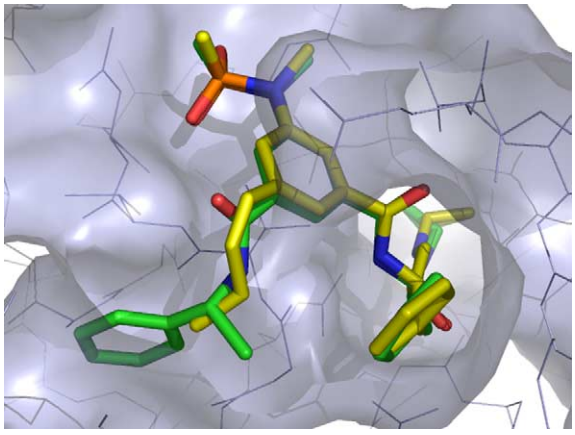


Figure 1. Overlay of **1** (green) with a model of **7m** (yellow) depicting S3 access using a conformationally defined olefin.

measurement. It should be noted that **7m** still contains a hydroxyethyl amine moiety, an amide bond, and a sulfonamide, all contributors to P-gp efflux.

In conclusion, we have demonstrated that the P2/P3 amide is non-critical for BACE-1 activity. The amide functionality acts mainly to orient the P3 ligand and the amide hydrogen bonding contacts do not significantly contribute to the binding energy. This was demonstrated through the synthesis of compounds that are conformationally biased to access S3. The ability to replace amide linkages with more hydrophobic homologs results in compounds possessing both lower polar surface area and less hydrogen bond donors and acceptors, two critical properties that contribute to P-gp efflux.

Acknowledgment

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